the growth in granule diameter (to 250% of control) was accompanied by a strong increase in the prevalence of capacitance flicker (to 41.5%), while in RBL-1 cells with granule size of 170% of control, capacitance flicker occured in 41.5% of cases after 5HT loading. These results suggest that granule size may influence fusion pore kinetics e.g. as a result of membrane curvature favoring rapid pore expansion.

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Cyclin Dependent Kinase 2 (CDK2) Regulates Secretory Function in Pancreatic Beta-Cells

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Cyclin dependent kinase 2 (CDK2) is a well-known regulator of the cell cycle in diverse types of cells. In pancreatic β-cells, CDKs have been the subject of much research devoted towards increasing β-cell mass by driving proliferation in diabetics, where there is a conspicuous loss of β-cell mass. However, mice lacking CDK2 in their pancreatic β-cells (Pdx1-Cre:CDK2$^{-/-}$ mice) exhibited glucose intolerance and reduced insulin release from isolated islets before losing β-cell mass. To examine the underlying defects, optical imaging of NAD(P)H/flavin autofluorescence was used to directly measure glycolytic and mitochondrial activity, which both trigger and control refilling of the readily-releasable granule pool in β-cells. By comparison to control islets, Pdx1-Cre:Cdk2$^{-/-}$ islets exhibited a reduced baseline, slower rise time, and a reduced maximal NAD(P)H/flavin response, which correlated with increased mitochondrial volume in EM images. Surprisingly, Ca^{2+} signaling - the direct trigger for β-cell insulin granule release - was not responsible for the observed secretory defect since the intracellular Ca^{2+} oscillations of Pdx1-Cre:Cdk2$^{-/-}$ islets were found to be more sensitive to glucose than control islets. Ca^{2+} channel activation is therefore uncoupled from both metabolism and secretion in this model. Uncoupling of secretion may further involve the exocytotic machinery, since mRNA levels of the SNARE protein SNAP25, and the glucose-dependent priming factor RAB27A, were reduced by CDK2 ablation. Taken together, our data indicates that CDK2 maintains insulin secretion by regulating metabolic output and plasma membrane electrical activity, and suggests further CDK2-dependent regulation of the exocytotic machinery, roles that are in distinct to regulation of β-cell proliferative capacity. Supported by F32DK085960 (MJM), the NIDDK Intramural Research Program (SGR), and R01DK46409 (LSS).

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Cholesterol Regulates Insulin Secretion through Protein Prenylation and Membrane Arrangement in INS-1E Cells

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Cholesterol plays a key role in membrane structure and secretory function of pancreatic B cells. Prenyl molecules derived from the cholesterol biosynthesis pathway, such as geranylgeranyl pyrophosphate (GGPP), serve as substrates for post-translational modifications of small GTPases involved in insulin secretion. However, appropriate cholesterol content is required for the maintenance of the membrane physicochemical properties such as fluidity that directly impact insulin granule fusion. Since statins reduce both prenyl groups and cholesterol, our aim was to study the relative contribution of these lipid lowering compounds on glucose-stimulated insulin secretion (GSIS) in insulin secreting cells.

INS-1E cells were cultured in the absence or presence of simvastatin (SIM, 1μM) or zaragozic acid (ZGA, inhibitor of cholesterol synthesis but not of prenyl groups, 20μM), in acute (2h) and chronic (24h) treatments; rat islets were acutely treated with SIM. To evaluate the role of protein prenylation, cells were treated for 24h with GGPP (20μM). Secreted insulin during 2h incubation at 2.8 and 16.7mM glucose was quantified by radioimmunoassay. Cellular cholesterol after treatment was measured by a colorimetric assay. Membrane lipid rafts after 2h SIM treatment were analyzed by immunofluorescence. Plasma membrane fluidity was measured by EPR using 5-DSA as probe.

Acute SIM reduces GSIS, but does not affect total cholesterol content, nor membrane microdomain distribution or membrane fluidity. GSIS inhibition was not observed after acute ZGA treatment nor after acute SIM treatment on cells previously loaded with GGPP. Chronic SIM inhibits GSIS but enhances cholesterol reduction and increases plasma membrane fluidity. Our results show that protein prenylation contributes to GSIS and that plasma membrane cholesterol content may be a critical parameter for the maintenance of physicochemical properties that regulate cellular functions such as granule fusion and cargo exocytosis.

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The Mechanism of AMPA Receptor Regulation of Insulin Release in Pancreatic Beta Cells

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Insulin, mainly stored in secretory granules of pancreatic beta cells, is the major hormone regulating glucose metabolism by complex but highly-ordered mechanisms. Ionotropic glutamate receptors (iGluRs) are expressed in islets and insulinoma cells and involved in insulin secretion. However, the exact roles that iGluRs play in beta cells remain unclear. The aim of the present study was to elucidate the current controversy about the presence of iGluRs in beta cells and explore their functions in insulin secretion, taking advantage of multiple techniques, including whole-cell recording in the acute pancreatic tissue slice, single beta cell nested RT-PCR and total internal reflection fluorescence microscopy (TIRFM). Here, we demonstrated that GluR2-containing α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) were expressed in mouse beta cells. Glutamate application increased both cytosolic calcium and the number of docked insulin-containing granules, which resulted in augmentation of depolarization-induced exocytosis and high-glucose-stimulated insulin release. While glutamate application directly depolarized beta cells, it also induced an enormous depolarization when KATP channels were available. Glutamate application reduced the conductance of KATP channels and increased voltage oscillations. Moreover, actions of AMPARs were absent in Kir6.2 knock-out mice. The effects of AMPARs on KATP channels were mediated by cytosolic cGMP. Taken together, our experiments uncovered a novel mechanism by which AMPARs participate in insulin release.

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Spatiotemporal Confinement of GLUT4 in Plasma Membrane Domains

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Translocation of glucose transporter-4 (GLUT4) to the plasma membrane (PM) is the hallmark of insulin regulation of glucose metabolism. However, GLUT4 translocation to PM proceeds through two distinct exocytotic mechanisms: classical fusion with dispersal and fusion with retention in specialized GLUT4 domains (clusters). In this study we examined the properties of GLUT4 domains and investigated insulin effect on spatiotemporal organization of GLUT4 in PM of live adipose cells by direct microscopic observation of single monomers tagged with photoswitchable fluorescent protein.

Using live cell fluorescent Photo-Activation Localization Microscopy (fPALM), we tracked diffusion of GLUT4-EOS in PM, and measured lifetime of GLUT4 molecules trapped inside the domains. While dispersed GLUT4 monomers followed Brownian motion with diffusion coefficient of ~0.09 μm2/s, GLUT4 associated with domains exhibited constrained diffusion limited by the boundaries of the elongated domains (sized 60-240 nm). We next analyzed the rates of GLUT4 monomer association with and dissociation from the domains, and found that insulin stimulated the latter by ~3-fold, but did not influence the former. Interestingly, outside the domains GLUT4 monomers appeared to frequently collide, but did not form new domains. Rather, GLUT4 domain formation was observed exclusively upon GLUT4 exocytosis. Consistent with previous findings, insulin increased the probability of fusion with dispersal (bypassing domain formation), and decreased the rate of GLUT4 internalization from domains, thus further augmenting the total amount of GLUT4 in PM and increasing the relative fraction of dispersed GLUT4.

All together these three effects of insulin act synergistically to shift the distribution of PM GLUT4 from clustered to dispersed states and concomitantly increase the residency time of GLUT4 monomers at the cell surface. Thus, we propose that spatiotemporal confinement of GLUT4 in PM domains represents a novel kinetic mechanism for insulin regulation of glucose homeostasis.

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A Secretory Artificial Cell for Exocytosis

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The complexity of exocytosis has left the molecular details of the process unclear. We present a minimal, artificial secretory cell designed for amperometric studies of release of signalling molecules through the fusion pore of single vesicles. In replacement of SNARE-proteins, the cell model has been equipped with an analog composed of complimentary DNA constructs, one on the vesicle and one on the target membrane. The DNA constructs hybridize in a zipper-like fashion bringing about docking of the vesicles and following the addition of